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(54) **NOVEL VEGF-LIKE FACTORS**

(57) A novel human gene having a significant homology with a VEGF-C gene, a member of the VEGF family, has been isolated by the PCR method using primers designed based on the sequence of EST that is assumed to be homologous with the C-terminal region of the VEGF-C gene. Mouse and rat genes have been isolated based on the human gene isolated as above. A protein encoded by the above human gene has been isolated by introducing the gene into *Escherichia coli* and expressing it. The isolated protein and genes can be applied to, for example, gene therapy for the VEGF-D deficiency, wound healing, and promotion of collateral vessel formation. Furthermore, VEGF-D protein inhibitors can be used as a novel anticancer drug, etc.

EP 0 935 001 A1

DescriptionTechnical Field

5 [0001] The present invention relates to a protein factor involved in angiogenesis in humans and falls in the field of genetic engineering.

Background Art

10 [0002] The process of angiogenesis, in which endothelial cells existing in the inner wall of blood vessels of animals generate new blood vessels, is triggered by transduction of a specific signal. A variety of substances are reportedly involved in this signal transduction. The most notable substance among them is the vascular endothelial growth factor (VEGF). VEGF is a protein factor which was isolated and purified, and can increase the proliferation of endothelial cells and the permeability of blood vessels (Senger, D. R. et al., Science 219: 983-985 (1983); Ferrara, N. and Henzel, W. J.,
15 Biochem. Biophys. Res. Commun. 161: 851-858 (1989)). It has been reported that the human VEGF gene contains eight exons and produces four subtypes consisting of 121, 165, 189, or 206 amino acid residues, depending on the difference in splicing, which causes different secretion patterns (Houck, K. A. et al., Mol. Endocrinol. 5: 1806-1814 (1991)). It has also been reported that there is a VEGF-specific receptor, fit-1, and that the binding of VEGF to fit-1 is important for the signal transduction (Vries, C. D. et al., Science 255: 989-991 (1992)).

20 [0003] Placental growth factor (PIGF) and platelet-derived growth factor (PDGF) have thus far been isolated and are factors related to VEGF. These factors are found to promote proliferation activities of vascular endothelial cells (Maglione, D. et al., Proc. Natl. Acad. Sci. USA 88: 9267-9271 (1991); Betsholtz, C. et al., Nature 320: 695-699 (1986)). In addition, VEGF-B (Olofsson, B. et al., Proc. Natl. Acad. Sci. USA 93: 2576-2581 (1996)) and VEGF-C (Lee, J. et al., Proc. Natl. Acad. Sci. USA 93: 1988-1992 (1996); Joukov, V. et al., EMBO J. 15, 290-299 (1996)) have recently been
25 isolated.

[0004] These factors appear to constitute a family, and this may contain additional unknown factors.

[0005] It has been suggested that VEGF is involved in not only vascular formation at the developmental stage but also in the pathological neovascularization associated with diabetes, rheumatoid arthritis, retinopathy, and the growth of solid tumors. Furthermore, in addition to its vascular endothelial cell growth-promoting effects listed above, VEGF's ability
30 to increase vascular permeability was suggested to be involved in the edema formation resulting from various causes. Also, these VEGF family factors may act on not only the blood vessels but also the blood cells and the lymphatic vessels. They may thus play a role in the differentiation and proliferation of blood cells and the formation of lymphatic vessels. Consequently, the VEGF family factors are presently drawing extraordinary attention for developing useful, novel drugs.

Disclosure of the Invention

[0006] An objective of the present invention is to isolate a novel protein belonging to the VEGF family and a gene encoding the protein. We searched for genes having homology to VEGF-C, which is a recently cloned VEGF family
40 gene, against Expressed Sequence Tags (EST) and Sequence Tagged Sites (STS) in the GenBank database. As a result, we found an EST that was assumed to have homology to the C-terminal portion of VEGF-C. We then designed primers based on the sequence, and amplified and isolated the corresponding cDNA using the 5' RACE method and the 3' RACE method. The nucleotide sequence of the isolated cDNA was determined, and the deduced amino acid sequence therefrom revealed that the amino acid sequence had significant homology to that of VEGF-C. Based on the
45 homology, we have assumed that the isolated human clone is a fourth member of the VEGF family (hereinafter designated as VEGF-D). We have also succeeded in expressing the protein encoded by the isolated human VEGF-D gene in E. coli cells, and have also purified and isolated it. Furthermore, we have succeeded in isolating the mouse and rat VEGF-D genes using the isolated human VEGF-D gene.

[0007] In particular, the present invention relates to a novel protein belonging to the VEGF family and a gene encoding
50 the protein. More specifically it relates to

- (1) A protein shown by SEQ ID NO.1 or having the amino acid sequence derived therefrom in which one or more amino acids are substituted, deleted, or added;
- (2) A protein encoded by a DNA that hybridizes with the DNA shown by SEQ ID NO. 2;
- (3) A DNA encoding the protein of (1);
- (4) A DNA hybridizing with the DNA shown by SEQ ID NO. 2;
- (5) A vector containing the DNA of (3) or (4);
- (6) A transformant carrying the vector of (5);

- (7) A method of producing the protein of (1) or (2), which comprises culturing the transformant of (6);
 (8) An antibody binding to the protein of (1) or (2);
 (9) A method of screening a compound binding to the protein of (1) or (2), which comprises a step of detecting the activity of the protein of (1) or (2) to bind to a test sample; and
 5 (10) A compound binding to the protein of (1) or (2), wherein said compound has been isolated by the method of (9).

[0008] The protein of the present invention (VEGF-D) has significant homology to VEGF-C and can be considered to be a fourth factor of the VEGF family. Since the major function of VEGF is vascular formation at the developmental stage and VEGF is considered to be involved in the pathological neovascularization associated with diabetes, rheumatoid arthritis, retinopathy, and the growth of solid tumors, the protein of the present invention is thought to have similar functions.

[0009] A person skilled in the art could prepare functionally equivalent proteins through modifying VEGF-D of the present invention by adding, deleting, or substituting one or more of the amino acids of VEGF-D shown by SEQ ID NO. 1 using known methods. Modifications of the protein can also occur naturally in addition to the artificial modifications described above. These modified proteins are also included in the present invention. Known methods for adding, deleting, or substituting amino acids include the overlap extension polymerase chain reaction (OE-PCR) method (Gene, 1989, 77 (1): 51).

[0010] The DNA encoding VEGF-D of the present invention, shown by SEQ ID NO. 2, is useful for isolating DNAs encoding the proteins having similar functions to VEGF-D in other organisms. For example, a person skilled in the art could routinely isolate homologs of human VEGF-D of the present invention from other organisms by allowing the DNA shown by SEQ ID NO. 2, or part thereof, as a probe, to hybridize with the DNA derived from other organisms. The DNA that hybridizes with the DNA shown by SEQ ID NO. 2 is also included in the present invention. The other organisms include mice, rats, and rabbits.

[0011] The DNA encoding a protein that is functionally equivalent to VEGF-D usually has high homology to the DNA shown by SEQ ID NO. 2. The high homology used herein means at least 70% or higher, more preferably 80% or higher, and still more preferably 90% or higher of sequence homology.

[0012] An example of the hybridization conditions for isolating the DNA having high homology will be given below. Prehybridization is performed in ExpressHyb Solution at 68°C for 30 minutes. The probe labeled with a radioisotope is denatured at 95°C to 100°C for 2 to 5 minutes and rapidly chilled on ice. The probe is added to a new ExpressHyb Solution. The blot is transferred to the solution containing the probe and allowed to hybridize under a temperature gradient of 68°C to 55°C for 2 hours. The blot is washed four times, for 10 minute each, with a 2 x SSC solution containing 0.05% SDS at room temperature. The blot is then washed with a 0.1 x SSC solution containing 0.1% SDS at 45°C for 3 minutes. The blot is subjected to autoradiography.

[0013] An example of the hybridization conditions for isolating the DNA having very high homology will be given below. Prehybridization is performed in ExpressHyb Solution at 68°C or 30 minutes. The probe labeled with a radioisotope is denatured at 95°C to 100°C for 2 to 5 minutes and rapidly chilled on ice. The probe is added into a new ExpressHyb Solution. The blot is transferred into the solution containing the probe, and allowed to hybridize at 68°C for 1 hour. The blot was washed four times, for 10 minute each, with a 2 x SSC solution containing 0.05% SDS at room temperature. The blot was then washed with a 0.1 X SSC solution containing 0.1% SDS at 50°C for 40 minutes, during which the solution was replaced once. The blot was then subjected to autoradiography.

[0014] Note that the hybridization condition can vary depending on the length of the probe (whether it is an oligomer or a probe with more than several hundred bases), the labeling method (whether the probe is radioisotopically labeled or non-radioisotopically labeled), and the type of the target gene to be cloned. A person skilled in the art would properly select the suitable hybridization conditions. In the present invention, it is especially desirable that the condition does not allow the probe to hybridize with the DNA encoding VEGF-C.

[0015] The DNA of the present invention is also used to produce VEGF-D of the present invention as a recombinant protein. Specifically, the recombinant protein can be produced in large quantity by incorporating the DNA encoding VEGF-D (for example, the DNA shown by SEQ ID NO. 2) into a suitable expression vector, introducing the resulting vector into a host, and culturing the transformant to allow the recombinant protein to be expressed.

[0016] The vector to be used for producing the recombinant protein is not particularly restricted. However, vectors such as pGEMEX-1 (Promega) or pEF-BOS (Nucleic Acids Res. 1990, 18(17): p.5322) are preferable. Suitable examples of the host into which the vector is introduced include E. coli cells, CHO cells, and COS cells.

[0017] The VEGF-D protein expressed by the transformant can be purified by suitably combining purification treatments such as solubilization with a homogenizer or a sonicator, extraction by various buffers, solubilization or precipitation by acid or alkali, extraction or precipitation with organic solvents, salting out by ammonium sulfate and other agents, dialysis, ultrafiltration using membrane filters, gel filtration, ion exchange chromatography, reversed-phase chromatography, counter-current distribution chromatography, high-performance liquid chromatography, isoelectric

focusing, gel electrophoresis, or affinity chromatography in which antibodies or receptors are immobilized.

[0018] Once the recombinant protein is obtained, antibodies against it can be prepared using known methods. The known methods include preparing polyclonal antibodies by immunizing rabbits, sheep, or other animals with the purified protein, and preparing monoclonal antibodies from the antibody-producing cells of immunized mice or rats. These antibodies will make it possible to quantify VEGF. Although the antibodies thus obtained can be used as they are, it will be more effective to use the humanized antibodies to reduce the immunogenicity. The methods of humanizing the antibodies include the CDR graft method and the method of directly producing a human antibody. In the CDR Graft method, the antibody gene is cloned from the monoclonal antibody-producing cells and its antigenic determinant portion is transplanted into an existing human antibody. In the method of directly producing a human antibody, a mouse whose immune system has been replaced by the human immune system is immunized, similar to ordinary monoclonal antibodies. The VEGF-D protein or its antibody thus obtained can be administered into the body by subcutaneous injection or a similar method.

[0019] A person skilled in the art could screen compounds that bind to the protein of the present invention by known methods.

[0020] For example, such compounds can be obtained by making a cDNA library on a phage vector (such as λ gt11 and ZAP) from the cells expected to express the protein that binds to the protein of the present invention (such as lung, small intestine, and heart cells of mammals), expressing the cDNAs on LB-agarose, fixing the expressed proteins onto a filter, preparing the purified protein of the present invention as a biotin-labeled or a fusion protein with the GST protein, and reacting this protein with the above filter. The desired compounds could then be detected by west western blotting using streptavidin or an anti-GST antibody (Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ullrich, A., and Schlessinger, J. (1991) Cloning of P13 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases, *Cell* 65: 83-90). Another method comprises the following steps. First, express the protein of the present invention fused with the SRF binding domain or the GAL4 binding domain in yeast cells. Second, prepare a cDNA library which expresses cDNAs fused with the transcription activation domain of VP16 or GAL4 from the cells expected to express a protein that binds to the protein of the present invention. Third, introduce the cDNA into the above yeast cells. Fourth, isolate the library-derived cDNA from the positive clones. Finally, introduce the isolated cDNA into *E. coli* to allow it to be expressed. (When a protein that binds to the protein of the present invention is expressed in yeast cells, the reporter gene is activated and the positive clone can be detected.) This method can be performed using the two-hybrid system (MATCHMAKER Two-Hybrid system, Mammalian MATCHMAKER Two-Hybrid Assay Kit, or MATCHMAKER One-Hybrid System (all by Clontech) or the HybriZAP Two-Hybrid Vector System (Stratagene) (Dalton, S. and Treisman, R. (1992) Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element, *Cell* 68: 597-612). Alternatively, the binding proteins can be screened by preparing a cDNA library from the cells expected to express a substance, such as a receptor, which binds to the protein of the present invention (for example, vascular endothelial cells, bone marrow cells, or lymph duct cells), introducing it into such cells as COS, detecting the binding of the protein of the present invention by itself or labeled with a radioisotope or a fluorescence, and cloning proteins that bind to the protein of the present invention (Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T., and Kishimoto, T. (1988) Cloning and expression of human interleukin-6 (BSF-2/IFN beta2) receptor, *Science* 241: 825-828, Fukunaga, R., Ishizaka-Ikeda, E., Seto, Y., and Nagata, S. (1990) Expression cloning of a receptor for murine granulocyte colony-stimulating factor, *Cell* 61: 341-350). Still another method comprises applying the culture supernatant or the cellular extract of the cells expected to express a protein that binds to the protein of the present invention onto an affinity column to which the protein of the present invention has been immobilized, and purifying the proteins specifically bound to the column. In addition, a DNA encoding the protein that binds to the protein of the present invention can be obtained by determining the amino acid sequence of the binding protein, synthesizing oligonucleotides based on the sequence, and screening a cDNA library with the oligonucleotides as probes.

[0021] Furthermore, compounds that bind to the protein of the present invention can be screened by contacting compounds, a natural substance bank, or a random phage peptide display library with the immobilized protein of the present invention and detecting the molecules bound to the protein. These compounds can also be screened by high throughput screening utilizing combinatorial chemistry technology (Wrighton, N. C., Farrell, F. X., Chang, R., Kashyap, A. K., Barbone, F. P., Mulcahy, L. S., Johnson, D. L., Barrett, R. W., Jolliffe, L. K., and Dower, W. J., Small peptides as potent mimetics of the protein hormone erythropoietin, *Science* (United States) Jul 26 1996, 273: 458-464, Verdine, G.L., The combinatorial chemistry of nature, *Nature* (England) Nov 7 1996, 384: 11-13, Hogan, J.C. Jr. Directed combinatorial chemistry, *Nature* (England) Nov 7 1996, 384: 17-19).

[0022] VEGF-D of the present invention may be used for gene therapy by introducing the VEGF-D gene into the body of the patient with the VEGF-D deficiency, or expressing the gene in the body. An anti-sense DNA of the VEGF-D gene may also be used to inhibit the expression of the gene itself, thereby suppressing the pathological neovascularization.

[0023] Among the many available methods to introduce the VEGF-D gene or its antisense DNA into the body, the retrovirus method, the liposome method, the cationic liposome method, and the adenovirus method are preferable.

[0024] In order to express these genes in the body, the genes can be incorporated into a suitable vector and introduced into the body by the retrovirus method, the liposome method, the cationic liposome method, or the adenovirus method. Although the vectors to be used are not particularly limited, such vectors as pAdexlcw and pZIPneo are preferable.

5 [0025] The present invention may also be applied for diagnosing disorders caused by abnormalities of the VEGF-D gene, for example, by PCR to detect an abnormality of the nucleotide sequence of the VEGF-D gene.

[0026] Furthermore, according to the present invention, the VEGF-D protein or its agonists can be used to heal wounds, promote collateral vessel formation, and aid hematopoiesis by the hematopoietic stem cells, by taking advantage of the angiogenic effect of the VEGF-D protein. The antibodies against the VEGF-D protein or its antagonists can be used as the therapeutic agents for pathological neovascularization, lymphatic dysplasia, dyshematopoiesis, or edemas arising from various causes. The anti-VEGF-D antibodies can be used for diagnosing diseases resulting from abnormal production of VEGF-D by quantifying VEGF-D.

Brief Description of the Drawings

15

[0027]

Figure 1 shows the relationship among the VEGF-D gene, the EST sequences, and the primers used for cloning.

Figure 2 compares the amino acid sequences of EST (H24828) and VEGF-C.

20 Figure 3 compares the amino acid sequences deduced from the VEGF-D gene and from the known genes of the VEGF family proteins.

Figure 4a shows the hydrophobicity plot of VEGF-D. Figure 4b shows the prediction of the cleavage site of the VEGF-D signal peptide.

Best Mode for Implementing the Invention

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[0028] The following examples illustrate the present invention in detail, but are not to be construed to limit the scope of the invention.

Example 1. Homology search by TFASTA method

30

[0029] The sequence CGPNKELDENTCQCVC (SEQ ID NO. 3) was designed based on the consensus sequence found in the BR3P (Balbiani ring 3 protein) repeat at the C-terminus of VEGF-C. The entire ESTs and STS sequences in the Genbank database (as of 29 February 1996) were then searched by the TFASTA method (Person and Lipman, Proc. Natl. Acad. Sci. USA 85: 2444-2448 (1988)). The searching conditions used are shown below (Table 1).

35

Table 1

40

Sequences	392,210
Symbols	135,585,305
Word Size	2
Gap creation penalty	12.0
Gap extension penalty	4.0

45

[0030] As a result, an EST (Accession No. H24828) that is considered to code the consensus sequence was found. The sequence is one of the ESTs registered by The WashU-Merck EST Project, and nine out of 16 amino acid residues were identical. Further searching for UniGene by NCBI based on this sequence revealed that five registered sequences (T64149, H24780, H24633, H24828, and T64277 (as of 1 March 1996)), including the above EST, were considered to be derived from the same gene. T64277 and T64149, as well as H24828 and H24780, are the combination of the 5' sequence and the 3' sequence of the same clones, and the length of the insert in both of these clones was 0.9 kb (Fig. 1).

55

[0031] Translating the H24828 sequence into a protein sequence in a frame where homology is found suggested that this sequence codes 104 C-terminal amino acid residues. Comparing this amino acid sequence with the C-terminus of VEGF-C, 28 out of 104 amino acids (27%) were identical. Moreover, the amino acids that are important for maintaining the protein structure, such as cysteine and proline, were well conserved (Fig. 2). Conserved sequences are shown in a

black box.

Example 2. cDNA cloning from a library

[0032] Primers for 5' RACE and 3' RACE (5' RACE primer: 5'-AGGGATGGGGAACCTTGAACGCTGAAT-3' (SEQ ID NO. 4), 3' RACE primer: 5'-GATCTAATCCAGCACCCCAAAACTGC-3' (SEQ ID NO. 5)) were designed (Fig. 1). A double-stranded cDNA was synthesized from human lung-derived polyA⁺ RNA using reverse transcriptase. PCR was then performed using Marathon-Ready cDNA, Lung (Clontech), having an adapter cDNA ligated to both ends as a template cDNA, and using the above primer and adapter primer (AP-1 primer) as primers. The above adapter cDNA contains the regions to which the adapter primers AP-1 and AP-2 hybridize. The PCR was performed in a manner such that the system was exposed to treatment at 94°C for 1 min; five cycles of treatment at 94°C for 30 sec and at 72°C for 4 min; five cycles of treatment at 94°C for 30 sec and at 70°C for 4 min; then 25 cycles of treatment at 94°C for 20 sec and at 68°C for 4 min. (TaKaRa Ex Taq (Takara Shuzo) and the attached buffer were used as Taq polymerase instead of Advantage KlenTaq Polymerase Mix.) As a result, 1.5kb fragments were amplified at the 5' region and 0.9kb fragments at the 3' region. These fragments were cloned with the pCR-Direct Cloning System (Clontech), CR-TRAP Cloning System (GenHunter), and PT7Blue-T vector (Novagen). When the 5'-RACE fragment was cloned into the pCR-Direct vector, the fragment was amplified again using 5'-CTGGTTCGGCCCAAGACTTGAACGCTGAATCA-3' (SEQ No. 7) and 5'-CTCGCTCGCCCACTAATACGACTCACTATAGG-3' (SEQ ID NO. 8) as primers.

Example 3. Nucleotide sequence analysis

[0033] ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase FS and 377 A DNA Sequencer (ABI) were used for DNA sequencing. The primers used are the primers in the vectors (5'-AATTAAC-CCTCACTAAAGGG-3' (SEQ ID NO. 9), 5'-CCAGGGTTTCCAGTCACGAC-3' (SEQ ID NO. 10)), AP-2 primer (5'-ACTCACTATAGGGCTCGAGCGGC-3' (SEQ ID NO. 11)), and 10 primers in the sequence shown below (Table 2).

Table 2

SQ1 (SEQ ID NO. 12)	5'-AAGTCTGGAGACCTGCT-3'
SQ2 (SEQ ID NO. 13)	5'-CAGCAGGTCTCCAGACT-3'
SQ3 (SEQ ID NO. 14)	5'-CGCACCCAAGGAATGGA-3'
SQ4 (SEQ ID NO. 15)	5'-TGACACCTGGCCATTCCA-3'
SQ5 (SEQ ID NO. 16)	5'-CATCAGATGGTAGTTCAT-3'
SQ6 (SEQ ID NO. 17)	5'-ATGCTGAGCGAGAGTCCATA-3'
SQ7 (SEQ ID NO. 18)	5'-CACTAGGTTTTCGGCAACTT-3'
SQ8 (SEQ ID NO. 19)	5'-GCTGTTGGCAAGCACTTACA-3'
SQ9 (SEQ ID NO. 20)	5'-GATCCATCCAGATCCCTGAA-3'
SQ10 (SEQ ID NO. 21)	5'-CAGATCAGGGCTGCTTCTA-3'

[0034] Determining the nucleotide sequence of the 1.5kb fragment at the 5'-side and the 0.9kb fragment at the 3'-side revealed that the sequence of the overlapping region was identical, confirming that 5'- and 3'-side cDNAs of the desired gene were obtained. Determining the entire nucleotide sequence of the cDNA revealed that this novel gene has the full length of 2 kb and can code a protein consisting of 354 amino acid residues (SEQ ID NO. 1 and SEQ ID NO. 2). Figure 1 shows the relation between this gene and the EST sequences registered in the Genbank database. Comparing the amino acid sequence with other VEGF family proteins revealed that the amino acids that are well conserved between family proteins are also conserved in this novel gene, and therefore this gene is obviously a new member of the VEGF family (Fig. 3). In Fig. 3, HSVEGF indicates human VEGF; HSVEGF-D, HSVEGF-C, and HSVEGF-B indicate human VEGF homologues (human VEGF-D, human VEGF-C, and human VEGF-B, respectively); HSPDGF-A indicates human PDGF-A; HSPDGF-B indicates human PDGF-B; and HSP1GF2 indicates human P1GF2. The conserved sequences are shown in a black box. Since VEGF-D is highly homologous to VEGF-C that was cloned as the Flt4 ligand, it was presumed to be a ligand to a Flt-4-like receptor.

[0035] Deducing the signal peptide cleavage site (Fig. 4b) by hydrophobicity plot (Fig. 4a) and the method of von Heijne (von Heijne, G, Nucleic Acids Res. 14, 4683-4690(1986)), N-terminal 21 amino acid residues may be cleaved as

signal peptides, and they may also undergo additional processing like VEGF-C.

Example 4. Northern blot analysis

- 5 **[0036]** A 1kb fragment, which had been cut out by digestion with EcoRI from the 5'-fragment subcloned into pCR-Direct vector, was labeled with [α - 32 P]dCTP and used as a probe. Labeling was performed by random priming using Ready-to Go DNA labeling beads (Pharmacia). Hybridization was performed in ExpressHyb Hybridization Solution (Clontech) by the usual method using Multiple Tissue Northern (MTN) Blot-Human, Human II, Human Fetal, and Human Cell lines (Clontech). Significant expression was observed in lung, heart, and intestine. Weak expression was
10 observed in skeletal muscle, ovary, colon, and pancreas. The apparent molecular weight of the mRNA was 2.2 kb, and the cloned fragment seemed to be almost the full length of the gene.

Example 5. VEGF-D protein expression in E. coli

- 15 **[0037]** Two primers, 5'-TCCAGATCTTTTGGCGCAACTTTCTATGACAT-3' (SEQ ID NO. 22) and 5'-CAGGTGCACT-CAAACAGGCACTAATTCAGGTAC-3' (SEQ ID NO. 23), were synthesized to amplify the region corresponding to the 89th to 181st amino acid residues of human VEGF cDNA. The thus-obtained DNA fragment was digested with restriction enzymes BglII and Sall, and ligated using ligation kit II (Takara Shuzo Co., Ltd) to plasmid pQE42 ((QIAGEN), which had been digested with restriction enzymes BamHI and Sall. The resulting plasmid was introduced into E. coli
20 SG19003[pREP4] (QIAGEN), and a plasmid, which was obtained as designed without any mutation, was selected (pQE42-BS3). Plasmid pQE42-BS3 was introduced into E. coli BL21 (Invitrogen) and cultured in 10 ml of L Broth containing 100 mg/l bicucilline (ampicillin sodium for injection, Meiji Seika Kaisha, Ltd.). 200 ml of fresh L Broth was then inoculated with the culture. After incubation at 37°C for 1.5 hours, IPTG was added to 3 mM, and the culture was further incubated at 37°C for 5 hours. After cells were harvested, a protein was purified with a Ni-NTA column following the protocol of QIAexpress Typell kit.
25

Example 6. Expression of DHFR-VEGF-D fusion protein in E. coli

- 30 **[0038]** The region corresponding to the 89th to 181st amino acid residues of human VEGF cDNA was amplified with the same primers used in Example 5. The thus-obtained DNA fragment was digested with restriction enzymes BglI and Sall. The fragment was then ligated using ligation kit II (Takara Shuzo Co., Ltd.) to the plasmid pQE40 (QIAGEN), which had been digested with restriction enzymes BamHI and Sall. The resulting plasmid was introduced into E. coli SG19003[pREP4] (QIAGEN), and a plasmid, which was obtained as designed without any mutation, was selected (pQE40-BS3). Plasmid pQE40-BS3 was introduced into E. coli BL21 (Invitrogen) and cultured in 10 ml of L Broth containing 100 mg/l bicucilline (ampicillin sodium for injection, Meiji Seika Kaisha, Ltd.). 200 ml of fresh L Broth was then
35 inoculated with the culture. After incubation at 37°C for 1.5 hours, IPTG was added to 3mM, and the culture was further incubated at 37°C for 5 hours. After cells were harvested, a DHFR-VEGF-D fusion protein was purified with a Ni-NTA column following the protocol of a QIAexpress Typell kit.

40 Example 7. Cloning mouse VEGF-D cDNA

- [0039]** Two Hybond-N+ (Amersham) filters (20 cm x 22 cm) on which 1.5×10^5 pfu of Mouse lung 5'-stretch cDNA library was transferred were prepared. Gradient hybridization from 68°C to 55°C was performed for 2 hours in ExpressHyb Hybridization Solution (Clontech) using as a probe an approximately 50 ng Pvu II fragment of human
45 VEGF-D, which had been labeled with α - 32 P-dCTP (Amersham) using Ready-To-Go DNA Labeling Beads(-dCTP) (Pharmacia). The filters were washed four times in 2 x SSC, 0.05% SDS at room temperature for 10 min, then washed in 0.1 x SSC, 0.1% SDS at 45°C for 3 min. The washed filters were exposed overnight at -80°C using HyperFilm MP (Amersham) and intensifying paper. Positive clones were subjected to the second screening in the same manner as above to isolate a single clone. Isolated lambda DNAs were purified from the plate lysate using a QIAGEN Lambda
50 MAX I Kit (Qiagen). Insert DNAs were cut out with EcoRI and subcloned into pUC118 EcoRI/BAP (Takara Shuzo Co., Ltd.). Its nucleotide sequence was then determined with ABI377 sequencer (Perkin Elmer). The cDNA coding the full length of mouse VRGF-D was reconstructed with two of the obtained clones that overlapped each other. SEQ ID NO. 24 shows the nucleotide sequence of mouse VEGF-D cDNA and the deduced amino acid sequence therefrom.

55 Example 8. Cloning rat VEGF-D cDNA

- [0040]** Two Hybond-N+ (Amersham) filters (20 cm x 22 cm), on which 1.5×10^5 pfu of Rat lung 5'-stretch cDNA library had been transferred, were prepared. Gradient hybridization from 68°C to 55°C was performed for 2 hours in

ExpressH.Fyb Hybridization Solution (Clontech) using as a probe an approximately 1 µg fragment containing 1-782 bp of the mouse VEGF-D cDNA which had been labeled with $\alpha^{32}\text{P}$ -dCTP (Amersham) using Ready-To-Go DNA Labeling Beads(-dCTP) (Pharmacia). The filters were washed four times in 2 x SSC, 0.05% SDS at room temperature for 10 min, then washed in 0.1 x SSC, 0.1% SDS at 45°C for 3 min. The washed filters were exposed overnight at -80°C using HyperFilm MP (Amersham) and intensifying paper. Positive clones were subjected to the second screening in the same manner as above to isolate a single clone. The isolated positive clone was excised into pBluescript using E. coli SOLAR (Stratagene) and helper phage ExAssist (Stratagene), then the sequence was determined with ABI377 sequencer (Perkin Elmer). The sequence seemed to be the rat VEGF-D cDNA but did not contain the termination codon.

[0041] To obtain the C-terminal cDNA which had not been obtained, PCR was performed using Marathon-Ready rat kidney cDNA (Clontech) as a template and 5' primer GCTGCGAGTGTGTCTGTAAA (SEQ ID NO. 26) and 3' primer GGGTAGTGGGCAACAGTGACAGCAA (SEQ ID NO. 27) with 40 cycles of 94°C for 15 sec, 55°C for 30 sec, and 72 °C for 2 min. After the thus-obtained fragment was subcloned into pGEM-T vector (promega), the nucleotide sequence was determined with ABI377 sequencer (Perkin Elmer). The resulting clone contained the C-terminus of rat VEGF-D. Based on the results of sequencing the clone obtained by plaque hybridization and the clone obtained by PCR, the full length of the rat VEGF-D sequence was determined. SEQ ID NO. 25 shows the determined nucleotide sequence and the deduced amino acid sequence therefrom.

Industrial Applicability

[0042] In the present invention, a novel protein (VEGF-D) having significant homology to VEGF-C and its gene have been isolated. VEGF-D appears to be involved in the pathological neovascularization associated with diabetes, rheumatoid arthritis, the growth of solid tumors, differentiation and proliferation of blood cells, formation of lymphatic vessels, and formation of edema resulting from various causes as well as the normal neovascularization at the developmental stage. The gene of the present invention can be used to diagnose disorders caused by abnormalities of the VEGF-D gene and gene therapy for the VEGF-D deficiency. The VEGF-D protein, which is obtained by expressing the gene of the present invention, can be used for healing wounds, promoting collateral vessel formation, and aiding hematopoietic stemcell proliferation. The antibodies or inhibitors against the VEGF-D protein can be used for treating angiodyplasia and lymphangiodysplasia associated with inflammation, edemas arising from various causes, dyshematopoiesis, and, as a novel anticancer agent, for treating pathological neovascularization. The VEGF-D protein and its antibodies can be useful for diagnosing diseases resulting from abnormal production of VEGF-D.

Sequence Listing

5 (1) Name or appellation of Applicant: Chugai Research Institute for
 Molecular Medicine, Inc.
 (2) Title of the Invention: Novel VEGF-like Factor
 (3) Reference Number: C1-802PCT
 10 (4) Application Number:
 (5) Filing date:
 (6) Country where the priority application was filed and the
 15 application number of the application: Japan, No. Hei 8-185216
 (7) Priority date: July 15, 1996
 (8) Number of sequences: 27

20 SEQ ID NO: 1
 SEQUENCE LENGTH: 354
 SEQUENCE TYPE: amino acid
 25 TOPOLOGY: linear
 MOLECULE TYPE: protein
 ORIGINAL SOURCE:
 ORGANISM: Homo sapiens
 30 TISSUE TYPE: lung
 SEQUENCE DESCRIPTION:
 Met Tyr Arg Glu Trp Val Val Val Asn Val Phe Met Met Leu Tyr Val
 1 5 10 15
 35 Gln Leu Val Gln Gly Ser Ser Asn Glu His Gly Pro Val Lys Arg Ser
 20 25 30
 Ser Gln Ser Thr Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser
 40 35 40 45
 Ser Leu Glu Glu Leu Leu Arg Ile Thr His Ser Glu Asp Trp Lys Leu
 50 55 60
 45 Trp Arg Cys Arg Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg
 65 70 75 80
 Ser Ala Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Ile
 85 90 95
 50 Glu Thr Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser
 100 105 110

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EP 0 935 001 A1

Pro Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr
115 120 125
5 Asn Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly
130 135 140
Cys Cys Asn Glu Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr
145 150 155 160
10 Ile Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro
165 170 175
Glu Leu Val Pro Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu
15 180 185 190
Pro Thr Ala Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln
195 200 205
20 Ile Pro Glu Glu Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile
210 215 220
Asp Met Leu Trp Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu
225 230 235 240
25 Asn Pro Leu Ala Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala
245 250 255
Leu Cys Gly Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val
260 265 270
30 Cys Lys Thr Pro Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys
275 280 285
Ser Cys Phe Glu Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His
35 290 295 300
Lys Leu Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe
305 310 315 320
40 His Thr Arg Pro Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys
325 330 335
Arg Phe Pro Lys Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys
340 345 350
45 Asn Pro

SEQ ID NO: 2

SEQUENCE LENGTH: 2004

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

5 ORIGINAL SOURCE:

ORGANISM: Homo sapiens

TISSUE TYPE: lung

10 FEATURE:

NAME/KEY: CDS

LOCATION: 403..1464

IDENTIFICATION METHOD: E

15 SEQUENCE DESCRIPTION:

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CCAGCTTCT GTARCTGTAA GCATTGGTGG CCACACCACC TCCTTACAAA GCAACTAGAA      60
CCTGCCGCAT ACATTGGAGA GATTTTTTTA ATTTCTGGA CAYGAAGTAA ATTTAGACTG      120
CTTTCYAATT TCAGGTAGAA GACATGTCCA CCTTCTGATT ATTTTGGAG AACATTTTGA      180
20 TTTTTTTCAT CTCTCTCTCC CCACCCCTAA GATTGTGCAA AAAAAGCGTA CCTTGCCTAA      240
TTGAAATAAT TTCATTGGAT TTTGATCAGA ACTGATCATT TGGTTTCTG TGTGAAGTTT      300
TGAGGTTTCA AACTTTCCTT CTGGAGAATG CCTTTTAAA CAATTTTCTC TAGCTGCCTG      360
25 ATGTCAACTG CTTAGTAATC AGTGGATATT GAAATATTCA AA ATG TAC AGA GAG      414
                                     Met Tyr Arg Glu
                                     1
TGG GTA GTG GTG AAT GTT TTC ATG ATG TTG TAC GTC CAG CTG GTG CAG      462
30 Trp Val Val Val Asn Val Phe Met Met Leu Tyr Val Gln Leu Val Gln
   5          10          15          20
GGC TCC AGT AAT GAA CAT GGA CCA GTG AAG CGA TCA TCT CAG TCC ACA      510
35 Gly Ser Ser Asn Glu His Gly Pro Val Lys Arg Ser Ser Gln Ser Thr
   25          30          35
TTG GAA CGA TCT GAA CAG CAG ATC AGG GCT GCT TCT AGT TTG GAG GAA      558
Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser Ser Leu Glu Glu
40          40          45          50
CTA CTT CGA ATT ACT CAC TCT GAG GAC TGG AAG CTG TGG AGA TGC AGG      606
Leu Leu Arg Ile Thr His Ser Glu Asp Trp Lys Leu Trp Arg Cys Arg
45          55          60          65
CTG AGG CTC AAA AGT TTT ACC AGT ATG GAC TCT CGC TCA GCA TCC CAT      654
Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg Ser Ala Ser His
50          70          75          80
CGG TCC ACT AGG TTT GCG GCA ACT TTC TAT GAC ATT GAA ACA CTA AAA      702
Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Ile Glu Thr Leu Lys

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EP 0 935 001 A1

	85		90		95		100	
	GTT ATA GAT GAA GAA TGG CAA AGA ACT CAG TGC AGC CCT AGA GAA ACG							750
5	Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro Arg Glu Thr							
		105		110		115		
	TGC GTG GAG GTG GCC AGT GAG CTG GGG AAG AGT ACC AAC ACA TTC TTC							798
10	Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr Asn Thr Phe Phe							
		120		125		130		
	AAG CCC CCT TGT GTG AAC GTG TTC CGA TGT GGT GGC TGT TGC AAT GAA							846
	Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly Cys Cys Asn Glu							
15		135		140		145		
	GAG AGC CTT ATC TGT ATG AAC ACC AGC ACC TCG TAC ATT TCC AAA CAG							894
	Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr Ile Ser Lys Gln							
		150		155		160		
20	CTC TTT GAG ATA TCA GTG CCT TTG ACA TCA GTA CCT GAA TTA GTG CCT							942
	Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu Leu Val Pro							
		165		170		175		180
25	GTT AAA GTT GCC AAT CAT ACA GGT TGT AAG TGC TTG CCA ACA GCC CCC							990
	Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu Pro Thr Ala Pro							
		185		190		195		
30	CGC CAT CCA TAC TCA ATT ATC AGA AGA TCC ATC CAG ATC CCT GAA GAA							1038
	Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Ile Pro Glu Glu							
		200		205		210		
	GAT CGC TGT TCC CAT TCC AAG AAA CTC TGT CCT ATT GAC ATG CTA TGG							1086
35	Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile Asp Met Leu Trp							
		215		220		225		
	GAT AGC AAC AAA TGT AAA TGT GTT TTG CAG GAG GAA AAT CCA CTT GCT							1134
	Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu Asn Pro Leu Ala							
40		230		235		240		
	GGA ACA GAA GAC CAC TCT CAT CTC CAG GAA CCA GCT CTC TGT GGG CCA							1182
	Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala Leu Cys Gly Pro							
45		245		250		255		260
	CAC ATG ATG TTT GAC GAA GAT CGT TGC GAG TGT GTC TGT AAA ACA CCA							1230
	His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val Cys Lys Thr Pro							
		265		270		275		
50	TGT CCC AAA GAT CTA ATC CAG CAC CCC AAA AAC TGC AGT TGC TTT GAG							1278
	Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys Ser Cys Phe Glu							

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EP 0 935 001 A1

280 285 290

5 TGC AAA GAA AGT CTG GAG ACC TGC TGC CAG AAG CAC AAG CTA TTT CAC 1326
Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His Lys Leu Phe His

295 300 305

10 CCA GAC ACC TGC AGC TGT GAG GAC AGA TGC CCC TTT CAT ACC AGA CCA 1374
Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe His Thr Arg Pro

310 315 320

15 TGT GCA AGT GGC AAA ACA GCA TGT GCA AAG CAT TGC CGC TTT CCA AAG 1422
Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys Arg Phe Pro Lys

325 330 335 340

GAG AAA AGG GCT GCC CAG GGG CCC CAC AGC CGA AAG AAT CCT 1464
Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys Asn Pro

20 345 350

25 TGATTCAGCG TTCCAAGTTC CCCATCCCTG TCATTTTAA CAGCATGCTG CTTTGCCAAG 1524
TTGCTGTCAC TGTTTTTTC CCAGGTGTTA AAAAAAAT CCATTTTACA CAGCACCACA 1584
GTGAATCCAG ACCAACCTTC CATTACACC AGCTAAGGAG TCCCTGGTTC ATTGATGGAT 1644

30 GTCTTCTAGC TGCAGATGCC TCTGCGCACC AAGGAATGGA GAGGAGGGGA CCCATGTAAT 1704
CCTTTTGTGT AGTTTGTGT TTGTTTTTG GTGAATGAGA AAGGTGTGCT GGTCATGGAA 1764
TGGCAGGTGT CATATGACTG ATTACTCAGA GCAGATGAGG AAAACTGTAG TCTCTGAGTC 1824
CTTGCTAAT CGCAACTCTT GTGAATTATT CTGATTCTTT TTTATGCAGA ATTTGATTCG 1884
TATGATCAGT ACTGACTTTC TGATTACTGT CCAGCTTATA GTCTTCCAGT TTAATGAACT 1944
ACCATCTGAT GTTTCATATT TAAGTGTATT TAAAGAAAAT AAACACCATT ATTCAAGTCT 2004

35 SEQ ID NO: 3
SEQUENCE LENGTH: 16
SEQUENCE TYPE: amino acid

40 TOPOLOGY: linear
MOLECULE TYPE: peptide
ORIGINAL SOURCE:

45 ORGANISM: Homo sapiens
TISSUE TYPE: lung
SEQUENCE DESCRIPTION:

50 Cys Gly Pro Asn Lys Glu Leu Asp Glu Asn Thr Cys Gln Cys Val Cys
1 5 10 15

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EP 0 935 001 A1

SEQ ID NO: 4
SEQUENCE LENGTH: 27
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
AGGGATGGGG AACTTGAAC GCTGAAT 27

15
SEQ ID NO: 5
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
25 GATCTAATCC AGCACCCCAA AAAGTGC 27

30
SEQ ID NO: 6
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
35 TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
40 CCATCCTAAT ACGACTCACT ATAGGGC 27

45
SEQ ID NO: 7
SEQUENCE LENGTH: 33
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
50 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CTGGTTCGGC CCAGAACTTG GAACGCTGAA TCA 33

55

SEQ ID NO: 8
SEQUENCE LENGTH: 32
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CTCGCTCGCC CACTAATACG ACTCACTATA GG 32

15 SEQ ID NO: 9
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
25 AATTAACCCT CACTAAAGGG 20

SEQ ID NO: 10
30 SEQUENCE LENGTH: 22
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
35 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CCAGGGTTTT CCCAGTCACG AC 22

40 SEQ ID NO: 11
SEQUENCE LENGTH: 23
45 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
50 SEQUENCE DESCRIPTION:
ACTCACTATA GGGCTCGAGC GGC 23

55

SEQ ID NO: 12
SEQUENCE LENGTH: 17
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
AAGTCTGGAG ACCTGCT 17

15 SEQ ID NO: 13
SEQUENCE LENGTH: 17
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
25 CAGCAGGTCT CCAGACT 17

30 SEQ ID NO: 14
SEQUENCE LENGTH: 17
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
35 TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
40 CGCACCCAAG GAATGGA 17

45 SEQ ID NO: 15
SEQUENCE LENGTH: 18
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
50 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
TGACACCTGG CCATTCCA 18

55

SEQ ID NO: 16
 SEQUENCE LENGTH: 18
 5 SEQUENCE TYPE: nucleic acid
 STRANDEDNESS: single
 TOPOLOGY: linear
 10 MOLECULE TYPE: other nucleic acid, synthetic DNA
 SEQUENCE DESCRIPTION:
 CATCAGATGG TAGTTCAT 18

15 SEQ ID NO: 17
 SEQUENCE LENGTH: 20
 SEQUENCE TYPE: nucleic acid
 20 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: other nucleic acid, synthetic DNA
 SEQUENCE DESCRIPTION:
 25 ATGCTGAGCG AGAGTCCATA 20

SEQ ID NO: 18
 30 SEQUENCE LENGTH: 20
 SEQUENCE TYPE: nucleic acid
 STRANDEDNESS: single
 35 TOPOLOGY: linear
 MOLECULE TYPE: other nucleic acid, synthetic DNA
 SEQUENCE DESCRIPTION:
 CACTAGGTTT GCGGCAACTT 20

40 SEQ ID NO: 19
 SEQUENCE LENGTH: 20
 45 SEQUENCE TYPE: nucleic acid
 STRANDEDNESS: single
 TOPOLOGY: linear
 50 MOLECULE TYPE: other nucleic acid, synthetic DNA
 SEQUENCE DESCRIPTION:
 GCTGTTGGCA AGCACTTACA 20

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EP 0 935 001 A1

SEQ ID NO: 20
SEQUENCE LENGTH: 20
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
GATCCATCCA GATCCCTGAA 20

15
SEQ ID NO: 21
SEQUENCE LENGTH: 19
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
25 CAGATCAGGG CTGCTTCTA 19

30
SEQ ID NO: 22
SEQUENCE LENGTH: 32
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
35 TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
TCCAGATCTT TTGCGGCAAC TTTCTATGAC AT 32

40
SEQ ID NO: 23
SEQUENCE LENGTH: 33
45 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
50 SEQUENCE DESCRIPTION:
CAGGTCGACT CAAACAGGCA CTAATTCAGG TAC 33

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EP 0 935 001 A1

SEQ ID NO: 24
SEQUENCE LENGTH: 1581
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
10 MOLECULE TYPE: cDNA to mRNA
ORIGINAL SOURCE:
ORGANISM: mouse
TISSUE TYPE: lung
15 FEATURE:
NAME/KEY: CDS
LOCATION: 96..1169
IDENTIFICATION METHOD: E
20 SEQUENCE DESCRIPTION:
TTCCGGGCTT TGCTGGAGAA TGCCTTTTGC AACACTTTTC AGTAGCTGCC TGGAAACAAC 60
TGCTTAGTCA TCGGTAGACA TTTAAAATAT TCAAA ATG TAT GGA GAA TGG GGA 113
25 Met Tyr Gly Glu Trp Gly
1 5
ATG GGG AAT ATC CTC ATG ATG TTC CAT GTG TAC TTG GTG CAG GGC TTC 161
Met Gly Asn Ile Leu Met Met Phe His Val Tyr Leu Val Gln Gly Phe
30 10 15 20
AGG AGC GAA CAT GGA CCA GTG AAG GAT TTT TCT TTT GAG CGA TCA TCC 209
Arg Ser Glu His Gly Pro Val Lys Asp Phe Ser Phe Glu Arg Ser Ser
35 25 30 35
CGG TCC ATG TTG GAA CGA TCT GAA CAA CAG ATC CGA GCA GCT TCT AGT 257
Arg Ser Met Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser Ser
40 40 45 50
TTG GAG GAG TTG CTG CAA ATC GCG CAC TCT GAG GAC TGG AAG CTG TGG 305
Leu Glu Glu Leu Leu Gln Ile Ala His Ser Glu Asp Trp Lys Leu Trp
55 60 65 70
CGA TGC CGG TTG AAG CTC AAA AGT CTT GCC AGT ATG GAC TCA CGC TCA 353
Arg Cys Arg Leu Lys Leu Lys Ser Leu Ala Ser Met Asp Ser Arg Ser
45 75 80 85
GCA TCC CAT CGC TCC ACC AGA TTT GCG GCA ACT TTC TAT GAC ACT GAA 401
Ala Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Thr Glu
50 90 95 100
55

EP 0 935 001 A1

	ACA CTA AAA GTT ATA GAT GAA GAA TGG CAG AGG ACC CAA TGC AGC CCT	449
	Thr Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro	
5	105 110 115	
	AGA GAG ACA TGC GTA GAA GTC GCC AGT GAG CTG GGG AAG ACA ACC AAC	497
	Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Thr Thr Asn	
10	120 125 130	
	ACA TTC TTC AAG CCC CCC TGT GTA AAT GTC TTC CGG TGT GGA GGC TGC	545
	Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly Cys	
	135 140 145 150	
15	TGC AAC GAA GAG GGT GTG ATG TGT ATG AAC ACA AGC ACC TCC TAC ATC	593
	Cys Asn Glu Glu Gly Val Met Cys Met Asn Thr Ser Thr Ser Tyr Ile	
	155 160 165	
20	TCC AAA CAG CTC TTT GAG ATA TCA GTG CCT CTG ACA TCA GTG CCC GAG	641
	Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu	
	170 175 180	
	TTA GTG CCT GTT AAA ATT GCC AAC CAT ACG GGT TGT AAG TGC TTG CCC	689
25	Leu Val Pro Val Lys Ile Ala Asn His Thr Gly Cys Lys Cys Leu Pro	
	185 190 195	
	ACG GGC CCC CGC CAT CCT TAC TCA ATT ATC AGA AGA TCC ATT CAG ACC	737
	Thr Gly Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Thr	
30	200 205 210	
	CCA GAA GAA GAT GAA TGT CCT CAT TCC AAG AAA CTC TGT CCT ATT GAC	785
	Pro Glu Glu Asp Glu Cys Pro His Ser Lys Lys Leu Cys Pro Ile Asp	
35	215 220 225 230	
	ATG CTG TGG GAT AAC ACC AAA TGT AAA TGT GTT TTG CAA GAC GAG ACT	833
	Met Leu Trp Asp Asn Thr Lys Cys Lys Cys Val Leu Gln Asp Glu Thr	
	235 240 245	
40	CCA CTG CCT GGG ACA GAA GAC CAC TCT TAC CTC CAG GAA CCC ACT CTC	881
	Pro Leu Pro Gly Thr Glu Asp His Ser Tyr Leu Gln Glu Pro Thr Leu	
	250 255 260	
45	TGT GGA CCG CAC ATG ACG TTT GAT GAA GAT CGC TGT GAG TGC GTC TGT	929
	Cys Gly Pro His Met Thr Phe Asp Glu Asp Arg Cys Glu Cys Val Cys	
	265 270 275	
	AAA GCA CCA TGT CCG GGA GAT CTC ATT CAG CAC CCG GAA AAC TGC AGT	977
50	Lys Ala Pro Cys Pro Gly Asp Leu Ile Gln His Pro Glu Asn Cys Ser	
	280 285 290	

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EP 0 935 001 A1

TGC TTT GAG TGC AAA GAA AGT CTG GAG AGC TGC TGC CAA AAG CAC AAG 1025
 Cys Phe Glu Cys Lys Glu Ser Leu Glu Ser Cys Cys Gln Lys His Lys
 5 295 300 305 310
 ATT TTT CAC CCA GAC ACC TGC AGC TGT GAG GAC AGA TGT CCT TTT CAC 1073
 Ile Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe His
 10 315 320 325
 ACC AGA ACA TGT GCA AGT AGA AAG CCA GCC TGT GGA AAG CAC TGG CGC 1121
 Thr Arg Thr Cys Ala Ser Arg Lys Pro Ala Cys Gly Lys His Trp Arg
 330 335 340
 15 TTT CCA AAG GAG ACA AGG GCC CAG GGA CTC TAC AGC CAG GAG AAC CCT 1169
 Phe Pro Lys Glu Thr Arg Ala Gln Gly Leu Tyr Ser Gln Glu Asn Pro
 345 350 355
 20 TGATTCAACT TCCTTTCAAG TCCCCCATC TCTGTCATTT TAAACAGCTC ACTGCTTGT 1229
 CAAGTTGCTG TCACTGTTGC CCACTACCCC TGCCCCCCCC CCCCCCGCC TCCAGGTGTT 1289
 AGAAAAGTTG ATTTGACCTA GTGTCATGGT AAAGCCACAT TTCCATGCAA TGGCGGCTAG 1349
 GTGATTCCCC AGTTCACTGA CAAATGACTT GTAGCTTCAA ATGTCTTTGC GCCATCANCA 1409
 25 CTCAAAAAGG AAGGGGTCTG AAGAACCCT TGTGTTGATAA ATAAAAACAG GTGCCTGAAA 1469
 CAAAATATTA GGTGCCACTC GATTGGGTCC CTCGGGCTGG CCAAATTCCA AGGGCAATGC 1529
 TCCTGAATTT ATTGTGCCCC TTCCTTAATG CGGAATTTCC TTTGTTTGA TT 1581

30 SEQ ID NO: 25
 SEQUENCE LENGTH: 1491
 SEQUENCE TYPE: nucleic acid
 35 STRANDEDNESS: double
 TOPOLOGY: linear
 MOLECULE TYPE: cDNA to mRNA
 ORIGINAL SOURCE:
 40 ORGANISM: rat
 TISSUE TYPE: lung
 FEATURE:
 45 NAME/KEY: CDS
 LOCATION: 270..1247
 IDENTIFICATION METHOD: E
 SEQUENCE DESCRIPTION:
 50 GCCACCTCTT GATTATTTGT GCAGCGGGAA ACTTTGAAAT AGTTTTCATC TCTTTCTCCC 60
 ATACTAAGAT TGTGTGTGGC CGTGGGGGAG TCCTTGACTA ACTCAAGTCA TTTCATTGGA 120

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EP 0 935 001 A1

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EP 0 935 001 A1

	Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu Leu Val	
	170 175 180	
5	CCT GTT AAA ATT GCC AAC CAT ACG GGT TGT AAG TGT TTG CCC ACG GGC	869
	Pro Val Lys Ile Ala Asn His Thr Gly Cys Lys Cys Leu Pro Thr Gly	
	185 190 195 200	
10	CCC CGG CAT CCT TAT TCA ATT ATC AGA AGA TCC ATT CAG ATC CCA GAA	917
	Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Ile Pro Glu	
	205 210 215	
15	GAA GAT CAA TGT CCT CAT TCC AAG AAA CTC TGT CCT GTT GAC ATG CTG	965
	Glu Asp Gln Cys Pro His Ser Lys Lys Leu Cys Pro Val Asp Met Leu	
	220 225 230	
20	TGG GAT AAC ACC AAA TGT AAA TGT GTT TTA CAA GAT GAG AAT CCA CTG	1013
	Trp Asp Asn Thr Lys Cys Lys Cys Val Leu Gln Asp Glu Asn Pro Leu	
	235 240 245	
25	CCT GGG ACA GAA GAC CAC TCT TAC CTC CAG GAA CCC GCT CTC TGT GGA	1061
	Pro Gly Thr Glu Asp His Ser Tyr Leu Gln Glu Pro Ala Leu Cys Gly	
	250 255 260	
30	CCA CAC ATG ATG TTT GAT GAA GAT CGC TGC GAG TGT GTC TGT AAA GCA	1109
	Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val Cys Lys Ala	
	265 270 275 280	
35	CCA TGT CCT GGA GAT CTC ATT CAG CAC CCG GAA AAC TGC AGT TGC TTT	1157
	Pro Cys Pro Gly Asp Leu Ile Gln His Pro Glu Asn Cys Ser Cys Phe	
	285 290 295	
40	GAA TGC AAA GAA AGT CTG GAA AGC TGT TGC CAA AAG CAC AAG ATG TTT	1205
	Glu Cys Lys Glu Ser Leu Glu Ser Cys Cys Gln Lys His Lys Met Phe	
	300 305 310	
45	CAC CCT GAC ACC TGC AGA TCA ATG GTC TTT TCA CTG TCC CCT	1247
	His Pro Asp Thr Cys Arg Ser Met Val Phe Ser Leu Ser Pro	
	315 320 325	
50	TAATTTGGTT TACTGGTGAC ATTTAAAGGA CATACTAACC TGATTTATTG GGGCTCTTTT	1307
	CTCTCAGGGC CCAAGCACAC TCTTAAAGGA ACACAGACGT TTGGCCTCTA AGAAATACAT	1367
	GGAAGTATTA TAGAGTGATG ATTAAATTGT CTTCTTGTTT CAAACAGGGT CTCATGATTA	1427
	CAGACCCGTA TTGCCATGCC TGCCGTCATG CTATCATGAG CGGAAAAGAA TCACTGGCAT	1487
	TTAA	1491

SEQ ID NO: 26
 SEQUENCE LENGTH: 20
 5 SEQUENCE TYPE: nucleic acid
 STRANDEDNESS: single
 TOPOLOGY: linear
 10 MOLECULE TYPE: other nucleic acid, synthetic DNA
 SEQUENCE DESCRIPTION:
 GCTGCGAGTG TGTCTGTAAA 20

15 SEQ ID NO: 27
 SEQUENCE LENGTH: 25
 SEQUENCE TYPE: nucleic acid
 20 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: other nucleic acid, synthetic DNA
 SEQUENCE DESCRIPTION:
 25 GGGTAGTG GGG CAACAGTGAC AGCAA 25

30

Claims

1. A protein shown by SEQ ID NO: 1 or having the amino acid sequence derived therefrom in which one or more amino acids are substituted, deleted, or added.
2. A protein encoded by a DNA hybridizing with the DNA shown by SEQ ID NO: 2.
3. A DNA encoding the protein of Claim 1.
4. A DNA hybridizing with the DNA shown by SEQ ID NO: 2.
5. A vector containing the DNA of Claim 3 or 4.
6. A transformant carrying the vector of Claim 5.
7. A method of producing the protein of Claim 1 or 2, which comprises culturing the transformant of Claim 6.
8. An antibody binding to the protein of Claim 1 or 2.
9. A method of screening a compound binding to the protein of Claim 1 or 2, which comprises a step of detecting the activity of the protein of Claim 1 or 2 to bind to a test sample.
10. A compound binding to the protein of Claim 1 or 2, wherein the compound have been isolated by the method of Claim 9.

Fig. 1

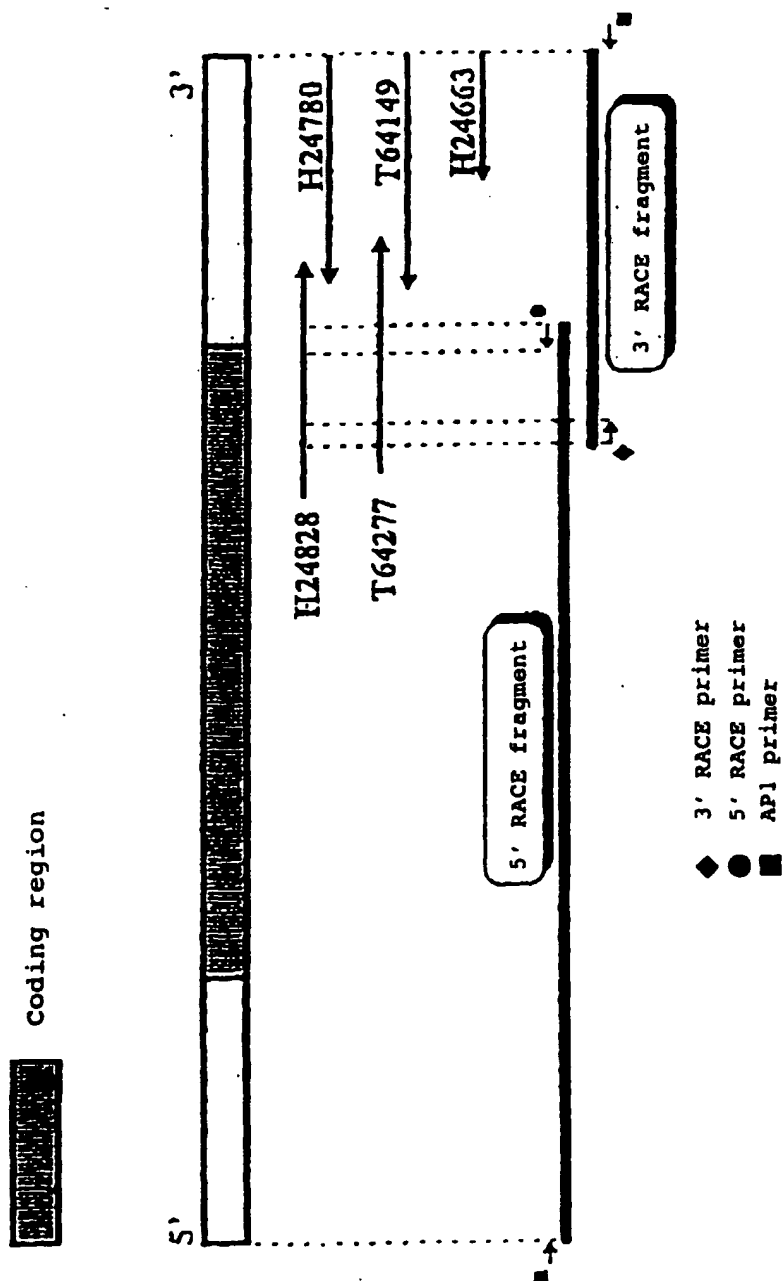


Fig. 2

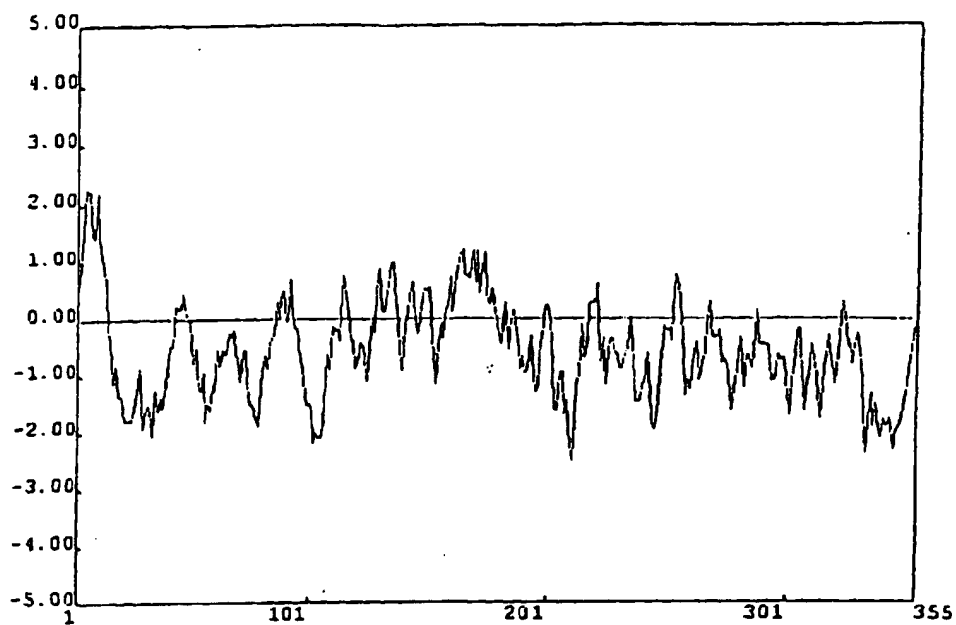
HSVEGFCC*	MHLLGFFSYA	CSLLAAALLP	GPREAPAAAA	AFESGLDLS	AEPDAGEATA	50
H24828	-----	-----	-----	-----	-----	50
HSVEGFCC	YASKDLEEQL	RSVSSVDELM	TVLYPEYWK	YKQLRKGGW	QHNREQANLN	100
H24828	-----	-----	-----	-----	-----	100
HSVEGFCC	SRTEETIKFA	AAHYNTEILK	SIDNEWRTQ	CMPREVCIDV	GKEFGVATNT	150
H24828	-----	-----	-----	-----	-----	150
HSVEGFCC	FFKPPCVSVY	RCGGCCNSEG	LOCMNTSTSY	LSKTLFEITV	PLSQGPKPVT	200
H24828	-----	-----	-----	-----	-----	200
HSVEGFCC	ISFANHTSCR	CMSKLDVYRO	VHSIIRSLP	ATLPQCQAAN	KTCPTNYMWN	250
H24828	-----	-----	-----	-----	-----	250
HSVEGFCC	NHICRCLAQE	DFMFSSDAGD	DSTDGFHDIC	GPNKELDEET	CQCVCRAGLR	300
H24828	-----	-----	-----	-----	-----HLQE	300
HSVEGFCC	PSGGPHKEL	ARNSSQVCS	NKLFPSCGA	NREFDENTQ	CVCKRTPRN	350
H24828	PSLGGPHMNF	EDRECVCS	TPCPKDLIQH	PKNCSCFEBK	ESLETCQKH	350
HSVEGFCC	QPLNIGKRA	CTESPQKCL	LKGGKFHHQT	CSYRPGTN	RQKAG-EPGF	400
H24828	KLFHDTSS	-----	-----DR	GPHTPGAS	GKTGAKHCR	400
HSVEGFCC	SYSVEVRCV	SYMRRQMS	450
H24828	FPKPKRAAQG	SHSRIN	450
*HSVEGFCC:	human VEGF-C					

Fig. 3

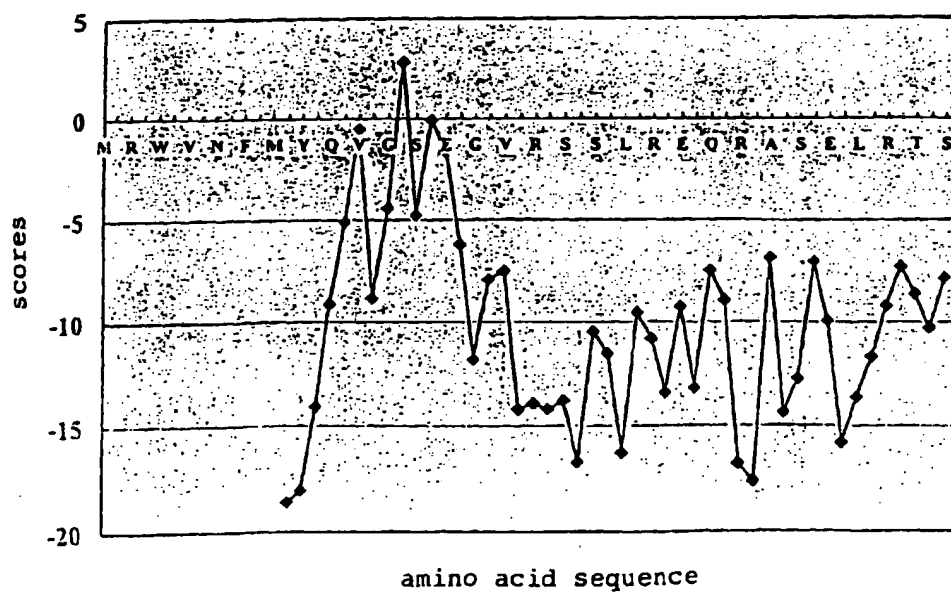
HSVEGF-D	YREWVVNV	FMMIYVQVVO	GSSNEHGPVK	-----	-----RSSO	50
HSVEGF-C	HLIGFESVA	CSLAAALP	GPREAPAAAA	AFESGLDLSO	AEPOAGEATA	50
HSPDGF-A	RTACILL	GCGYAHVUA	EEAEIPREVI	ERLAR-----	-----SQ	50
HSPDGF-B	NRCWAFLS	LCCYRLVSA	EGDPIPEELY	EMLSO-----	-----HS	50
HSPDGF2	PMVRLPCF	LQLLAGLAP	AVPPQWALS	AGNGS-----	-----	50
HSVEGF	NFILSWVHW	SLATLYTHH	AKWSQAAPHA	EGGGQ-----	-----	50
HSVEGF-B	SPILRR--	--LAAALQ	LAPAAQAPVSO	PDAPG-----	-----	50
HSVEGF-D	STLERSETOI	RAASLEELL	RITHSDAHL	WRRLRLKSF	TSMDSRSASH	100
HSVEGF-C	YAKDLEEQ	RSVSVDGLM	TVYYPYHM	YKQLRKGGW	QHNREQANLN	100
HSPDGF-A	IHSIRDLRI	LEIDVGSSE	S-----	-----	-----DTSLRA	100
HSPDGF-B	IRSFDDLRI	LHGDPGELOG	AEL-----	-----	-----DLNMTR	100
HSPDGF2	-----	-----	-----	-----	-----	100
HSVEGF	-----	-----	-----	-----	-----	100
HSVEGF-B	-----	-----	-----	-----	-----	100
HSVEGF-D	RST---RFA	ATFYDITL	VIDEEWQTO	SPRITCEV	ASLGKSLNT	150
HSVEGF-C	SRTEETKFA	SAHYNTIL	SDNEWRTQ	CMPEVCIQV	GKFGVAJNT	150
HSPDGF-A	HGVHAKHVP	KRPLPYRR	RSIEEAVPAY	KYIVYIYEI	PRSQVDPISA	150
HSPDGF-B	SHSGGEESL	ARGRSIGSL	TIAEPAMIAE	KYIEVEFEI	SRLIDRIINA	150
HSPDGF2	-----	---SEVVVP	FOEV-WGASY	RALRLQDV	VSYPSEVEH	150
HSVEGF	-----	---NHVVV	FMDV-YQASY	HTITLQDI	FOYYPDEIEY	150
HSVEGF-B	-----	---HQKXVS	WLV-YTAT	OPREVVPL	TVLMGTVAK	150
HSVEGF-D	FE--KPPCVN	FRGGGCE	SSLMNIST	SYISKLFEL	-SPLTSVPE	200
HSVEGF-C	FE--KPPCVS	YRGGGCS	GLDMNIST	SYLSKTLFEI	-TPLSQGPK	200
HSPDGF-A	NELIWPCCVE	KRYHCCMT	SSVQOASRV	HHRSVKVKY	EYKPKPKLK	200
HSPDGF-B	NELVWPCCVE	QSSSGCCMN	RNVQRRQV	QLRPVVRK	EYKPKPIFK	200
HSPDGF2	ML--SSSCVS	LLRTCCGD	INMHVET	ANVTMLLK	---SGDRPS	200
HSVEGF	IL--KSSCAP	LMRGGCCD	EGEVPIEE	SNITMIMR	---KPHGQGH	200
HSVEGF-B	QL--VSSAT	QRCCGCCPD	DGLEVPIGO	HQVRMILN	---GYPSSQ-	200
HSVEGF-D	LIPVKVANIT	GKGLT--A	PRHPYSIIR	SIQIPEEDC	SHSALCPTD	250
HSVEGF-C	PTISFANIT	SRRMSKLDV	YRQVHSIIR	S-LPATLPC	QAANATCPIN	250
HSPDGF-A	EQVRLEEIL	EAAATSLN	PDYREEDTG	P-RESGK-K	S--RLKPI	250
HSPDGF-B	KATVTLEDL	AKKET-VAA	ARPVTRSPG	S-DEORAL	-----	250
HSPDGF2	YELTFSQV	RERRE---	LREMKKPER	R-PKGRGGR	---FQORP-	250
HSVEGF	IGEMSFLQ-N	KERRE-KKO	RARQEKKSVE	G-KGKGQK	---FASRYK-	250
HSVEGF-B	LGEMSLEEIS	QERREKKKD	SA-----	-----	-----	250
HSVEGF-D	MLDSNKKK	VIDEENLA	GTEDSHLQE	-----	-----	300
HSVEGF-C	YMNHHIGRE	LAQDFRFS	DAGDOSTDGF	HDICGPNKEL	DEETQOEVR	300
HSPDGF-A	-----	-----	-----	-----	-----	300
HSPDGF-B	-----	-----	-----	-----	-----	300
HSPDGF2	-----	-----	-----	-----	-----	300
HSVEGF	-----	-----	-----	-----	-----	300
HSVEGF-B	-----	-----	-----	-----	-----	300
HSVEGF-D	----PALCOP	MMFEDDRGE	VCVTPCPKD	LIQHPKNCSC	FEKESL-EI	350
HSVEGF-C	AGLRPASCOP	KEIDRNSQ	VCVANKFPS	QCGANREFDE	NTQCVCRR	350
HSPDGF-A	-----	-----	-----	-----	-----	350
HSPDGF-B	VRVRRPPKPK	ARKFKHTDK	TALDETGA	-----	-----	350
HSPDGF2	-----	-----	-----	-----	-----	350
HSVEGF	N-TDSRCKAR	QLEINERTCR	CDKPRR...	-----	-----	350
HSVEGF-B	RRSFLRCQGR	GLEINPDTER	GRKLRR...	-----	-----	350
HSVEGF-D	CCQKHK-FHF	DTSCGE----	-----	---DRCPFHT	RFGASGKTAC	400
HSVEGF-C	CPRNOPI-NE	GKCACTES	PQCLLKGGK	FHHQTGSCYR	REGTNRQKAC	400
HSPDGF-A	-----	-----	-----	-----	-----	400
HSPDGF-B	-----	-----	-----	-----	-----	400
HSPDGF2	-----	-----	-----	-----	-----	400
HSVEGF	-----	-----	-----	-----	-----	400
HSVEGF-B	-----	-----	-----	-----	-----	400
HSVEGF-D	AKHCRFPK	RAAQGHSMR	NE...	-----	-----	450
HSVEGF-C	-EPGFSYSSE	VCRCVSYW	RQMS...	-----	-----	450
HSPDGF-A	-----	-----	-----	-----	-----	450
HSPDGF-B	-----	-----	-----	-----	-----	450
HSPDGF2	-----	-----	-----	-----	-----	450
HSVEGF	-----	-----	-----	-----	-----	450
HSVEGF-B	-----	-----	-----	-----	-----	450

Fig. 4

a) Hydrophobicity



b) Prediction of the human VEGF-D signal peptide



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/02456

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl ⁶ C12N15/18, C12N15/63, C12P21/02, C07K14/485, C07K16/22, G01N33/50 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int. Cl ⁶ C12N15/18, C12N15/63, C12P21/02, C07K14/485, C07K16/22, G01N33/50 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI, WPI/L, BIOSIS PREVIEWS, CAS ONLINE, GENETYX-MAC/CD		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	Yamada, Y. et al. "Molecular cloning of a novel vascular endothelial growth factor, VEGF-D." Genomics (1997, Jun.), Vol. 42, No. 3, p. 483-488	1 - 10
X	Vladimir, J. et al. "A novel vascular endothelial growth factor, VEGF-C, (VEGFR-2) receptor tyrosine kinases" EMBO J. (1996, Jan.) Vol. 15, No. 2, p. 290-298	1 - 2
X	Vladimir, J. et al. "A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4(VEGFR-3) and KDR(VEGFR-2) receptor tyrosine kinases" EMBO J. (1996, Jan.) Vol. 15, No. 7, p. 1751	1 - 2
PX	Maurizio, O. et al. "Identification of a c-fos-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family" Proc. Natl. Acad. Sci. USA (1996, Oct.) Vol. 93, p. 11675-11680	1 - 2
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "A" document member of the same patent family		
Date of the actual completion of the international search October 7, 1997 (07. 10. 97)		Date of mailing of the international search report October 21, 1997 (21. 10. 97)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/02456

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Georg. B. et al. "Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation" Development (1992) Vol. 114, p. 521-532	1 - 10
X	David, T.S. et al. "The mouse gene for vascular endothelial growth factor" J. Biol. Chem. (1996, Feb.) Vol. 271, No. 7, p. 3877-3883	1 - 10
X	Kevin, P.C. et al. "Vascular endothelial growth factor" J. Biol. Chem. (1992) Vol. 267, No. 23, p. 16317-16322	1 - 10
X	Greg, C. et al. "Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor" Proc. Natl. Acad. Sci. USA (1990) Vol. 87, p. 2628-2632	1 - 10
X	Edmund, T. et al. "The human gene for vascular endothelial growth factor" J. Biol. Chem. (1991) Vol. 266, No. 18, p. 11947-11954	1 - 10

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/02456

Disclosure other than written disclosures

1. The GenBank Database (Rel. 100) on GENETYX, Accession No. D89628, Yoshiki Yamada, Chugai Research Institute for Molecular Medicine. (29-Nov-1996)

2. The GenBank Database (Rel. 100) on GENETYX, Accession No. T64277, Hillier, L. et al. (1995)

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